Analysis of the Genetic Structure of the Populations of Marsh Frog (*Pelophylax Ridibundus*) in the Impact Territories of the City of Belgorod on the Basis of Microsatellite Markers of DNA

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Abstract—On the basis of SSR-method the genetic structure of marsh frog populations (*Pelophylax ridibundus*) in the urbanized area of Belgorod and its suburbs was studied. The data obtained indicate a high level of genetic variability in the populations studied. The heterozygosity level was equal to \(H_e=0.731\pm0.027\), \(H_o=0.587\pm0.037\), Shannon index - \(I=1.737\pm0.082\), and the effective number of alleles - \(A_e=4.992\pm0.364\). However, in a number of populations there is a trend towards a decrease in genetic diversity and an increase in the level of inbreeding.

Keywords—*Pelophylax ridibundus*, SSR, population, urbanized area

I. INTRODUCTION

Microsatellite markers have unrivalled information value as genetic markers of the population structure. The minimum amount and simplicity of tissue storage required for analysis, a large variety of individual loci, high allelic variability - all this has allowed microsatellite markers to find wide application in various fields of biology, both in classical genetics and ecology. Molecular markers can be used to evaluate important parameters such as mutagenesis, drift and gene flow, and effective population size. Microsatellites are especially useful in population studies because of their high polymorphism [1, 2].

Amphibians, as second and subsequent consumers, are a link in the trophic chains between the aquatic and terrestrial parts of biocoenoses. In addition, amphibians are very sensitive to changes in environmental factors, which makes them convenient bioindicators [3-5].

The purpose of the study was to analyze the genetic structure of marsh frog populations (*Pelophylax ridibundus* Pallas, 1771) in the urbanized landscape of Belgorod and its surroundings.

II. EXPERIMENTAL

The material was collected during the summer field season of 2016. A total of 67 animals from 6 locations were collected (Fig. 1): 1. «Severskiy Donets» (50°31'48.0"N 6°38'59.5"E); 4. «Sevryukovo» (50°36'55.3"N 36°46'21.9"E); 5. «Dubovoe» (50°32'00.3"N 36°34'59.8"E); 6. «Vezelka» (50°35'52.8"N 36°33'37.1"E). The points differed by the gradient of the anthropogenic press.

DNA was extracted from muscle tissue. To isolate genomic DNA, a set of DNA Extran-2 (Sintol) was used, according to the protocol proposed by the company. The obtained DNA solution was stored at -20°C.

DNA variability was analyzed using polymerase chain reaction by SSR-PCR (Simple Sequence Repeats) method. Samples were prepared per tube before amplification as follows: 2.5x Reaction mixture (2.5x PCR buffer B (KCl, TrisHCl (pH 8.8), 6.25 mM MgCl2), SynTaq DNA polymerase, deoxynucleoside triphosphates, glycerol, Tween 20 - 8 µl; MgCl2 25 mM - 0.5 µl; deionized water - 9.3 µl, mix of primers 0.05 µl.

The study used primers Res14, Res15, Res17, Res22, Rrid059A, Rrid082A [7].

PCR amplification was performed in the DNA amplifier Veriti (Thermo FS). PCR amplification involved an initial
cycle of denaturation at 95 °C for 15 min and 33 subsequent cycles of 94 °C for 30 s, 60 °C for 90 s, and 72 °C for 60 s, followed by a final extension step at 60 °C for 30 min [7]. PCR products were run on an ABI 3500 (Applied Biosystems) genetic analyzer with SD 450 (Sintol) size standard. The peaks were visualized using GeneMapper 3.7 (Applied Biosystems) software.

The obtained results were processed using standard methods of variation statistics [8]. Statistical processing was carried out using MS Excel, Statistica 6.0 and GenAlEx [9].

### III. RESULTS AND DISCUSSION

The results of the analysis are presented in tables I-II. According to the data obtained, 7 microsatellite loci contained 6 to 11 alleles, with an average value of 8.69 alleles per locus. The level of actual and expected heterozygosity was quite high (\(He=0.731\pm0.027\), \(Ho=0.587\pm0.037\)). The highest values of genetic diversity were noted in the items «Razumnaya-1» (\(He=0.848\)) and «Seversky Donets» (\(He=0.733\)). The highest indices of the Schenon index (I=2.162 and 1.842, respectively) are also noted here. In other populations, one can trace the trend towards a decrease in allelic diversity and a possible transition of loci to a homozygous state, which, in turn, may reduce the viability of these groups, since a certain level of genetic variability, being a "mobilization reserve", ensures the stability of the population as a system [10].

Value of the inbreeding coefficient is noted in the point «Dubovoe» (\(F=0.384\)) the maximum. The lowest actual heterozygosity index \(Ho=0.454\) is also fixed here.

According to the data presented in Table II, the genetic distance (D) calculated on the basis of Nei [11] between some populations turned out to be quite significant, which in turn confirms their high genetic differentiation.

The Principal Coordinates (PCoA) analysis based on the calculated genetic distances (Fig. 2) revealed the nature of genetic subdivision of the studied groups, which were distributed over three clusters.

The most original population was the «Razumnaya-2» population. In general, according to the Wright model, the

### TABLE I. INDICATORS OF THE GENETIC DIVERSITY OF THE POPULATIONS OF P. RIDIBUNDUS

<table>
<thead>
<tr>
<th>Point</th>
<th>N</th>
<th>Aa</th>
<th>Ae</th>
<th>I</th>
<th>Ho</th>
<th>He</th>
<th>F</th>
<th>P</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.«Seversky Donets»</td>
<td>14</td>
<td>11.0</td>
<td>4,502</td>
<td>1,842</td>
<td>0.751</td>
<td>0.733</td>
<td>-0.039</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>2.«Razumnaya -1»</td>
<td>11</td>
<td>11.0</td>
<td>7,423</td>
<td>2,162</td>
<td>0.687</td>
<td>0.848</td>
<td>0.198</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>3.«Razumnaya -2»</td>
<td>12</td>
<td>8.5</td>
<td>4,687</td>
<td>1,731</td>
<td>0.544</td>
<td>0.731</td>
<td>0.247</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>4.«Sevryukovo»</td>
<td>12</td>
<td>8.0</td>
<td>4,818</td>
<td>1,590</td>
<td>0.551</td>
<td>0.667</td>
<td>0.124</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>5.«Dubovoe»</td>
<td>9</td>
<td>7.0</td>
<td>4,383</td>
<td>1,580</td>
<td>0.454</td>
<td>0.707</td>
<td>0.384</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>6.«Vezelka»</td>
<td>10</td>
<td>6.5</td>
<td>4,143</td>
<td>1,518</td>
<td>0.537</td>
<td>0.702</td>
<td>0.287</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>The average</td>
<td>8,690</td>
<td>± 4,992</td>
<td>± 1,737</td>
<td>± 0.587</td>
<td>± 0.731</td>
<td>± 0.200</td>
<td>± 0.000</td>
<td>± 0.442</td>
<td>± 100.0</td>
</tr>
</tbody>
</table>
| Note: N – the number of individuals in the sample; Aa – the average number of alleles; Ae – the effective number of alleles; I – the Shannon index; Ho – observed heterozygosity; He – the expected heterozygosity; F – inbreeding coefficient, P – percentage of polymorphic loci.

### TABLE II. PAIRED ESTIMATES OF THE GENETIC DISTANCE BETWEEN POPULATIONS PELOPHYLAX RIDIBUNDUS

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>0,000</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0,520</td>
<td>0,000</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,305</td>
<td>1,429</td>
<td>0,000</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,975</td>
<td>2,687</td>
<td>3,311</td>
<td>0,000</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>1,623</td>
<td>1,980</td>
<td>2,498</td>
<td>0,236</td>
<td>0,000</td>
<td>5</td>
</tr>
<tr>
<td>2,964</td>
<td>2,473</td>
<td>2,747</td>
<td>0,255</td>
<td>0,236</td>
<td>0,000</td>
</tr>
</tbody>
</table>

![Fig. 2. Analysis of the principal coordinates.](image)

The subdivision index between the populations was not high – \(F_{st}=0.184\pm0.028\), and the gene flow index was \(Nm=1.3\pm0.2\) individuals per generation.

### IV. CONCLUSION

According to the analysis of the genetic structure of P. ridibundus based on microsatellite markers, a high level of genetic variability and weak genetic isolation is observed in most populations. Meanwhile, a number of groups have recorded a decrease in genetic diversity and an increase in inbreeding, which may be due to the impact of the anthropogenic press, in particular, the process of insulation, i.e. the fragmentation of populations into small isolated groups.

### REFERENCES


